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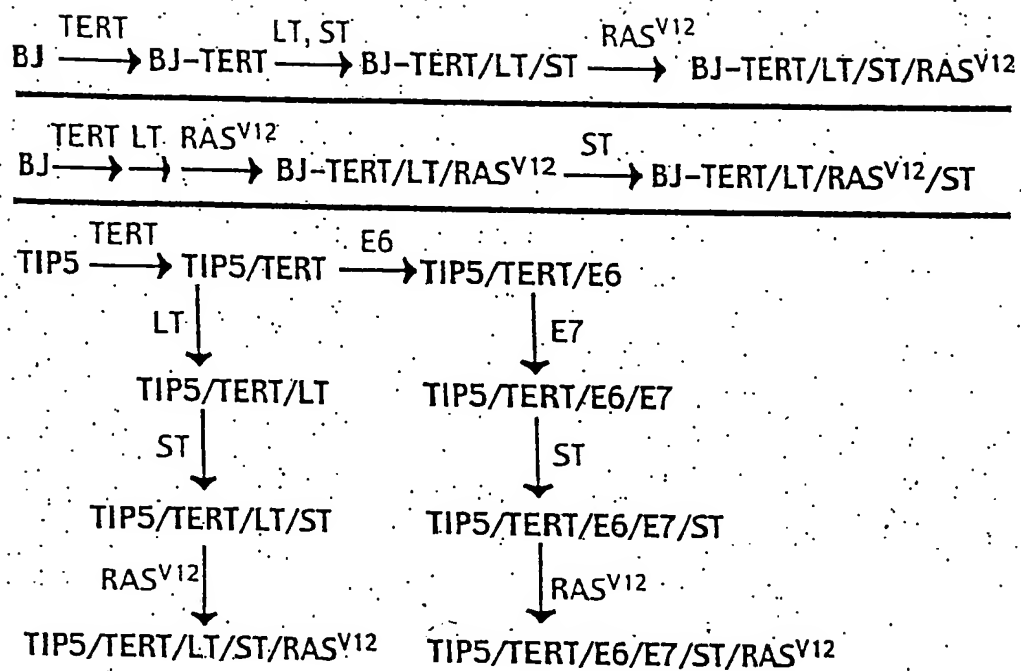
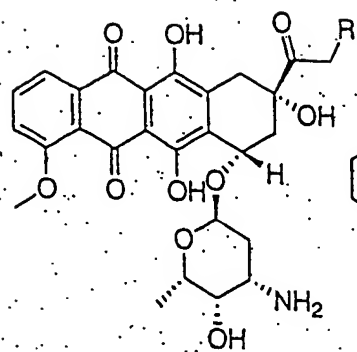
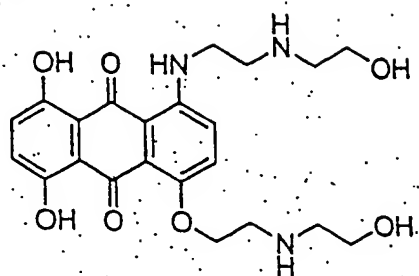


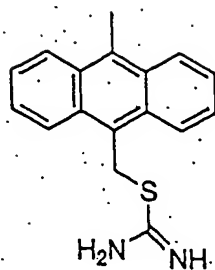
Figure 1



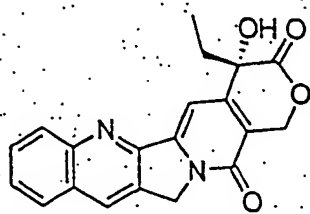
R = H, daunorubicin
R = OH, doxorubicin



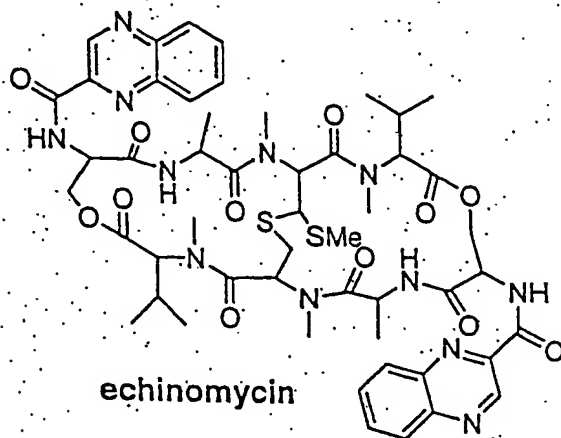
mitoxantrone



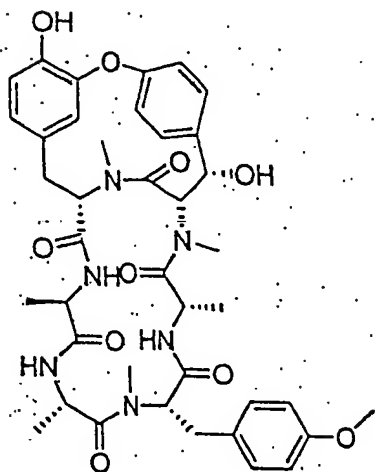
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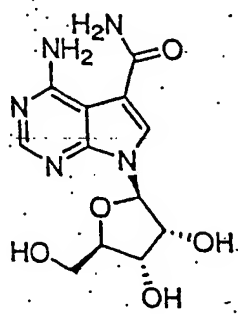
camptothecin



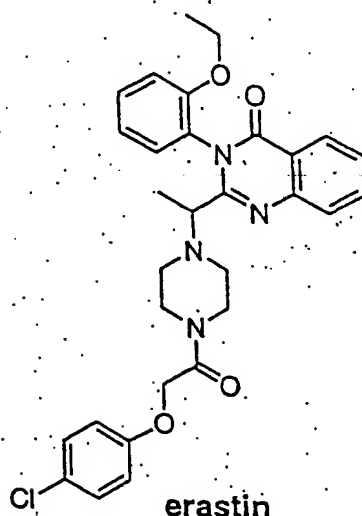
echinomycin



bouvardin



sangivamycin



erastin

Figure 2

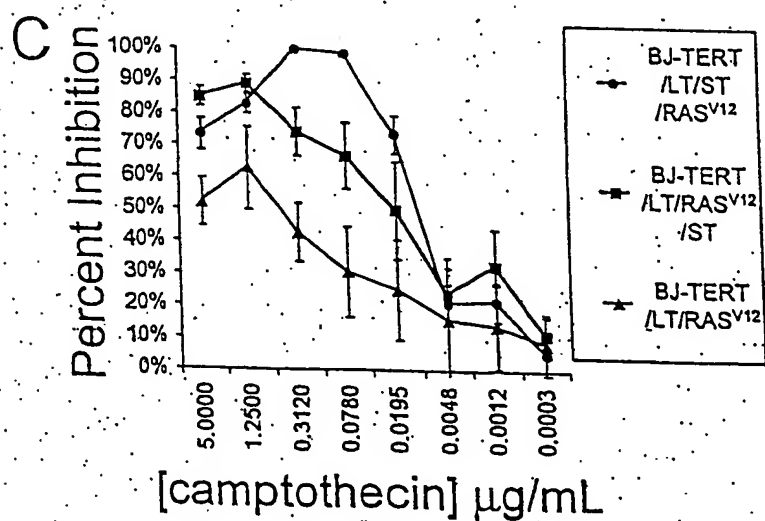
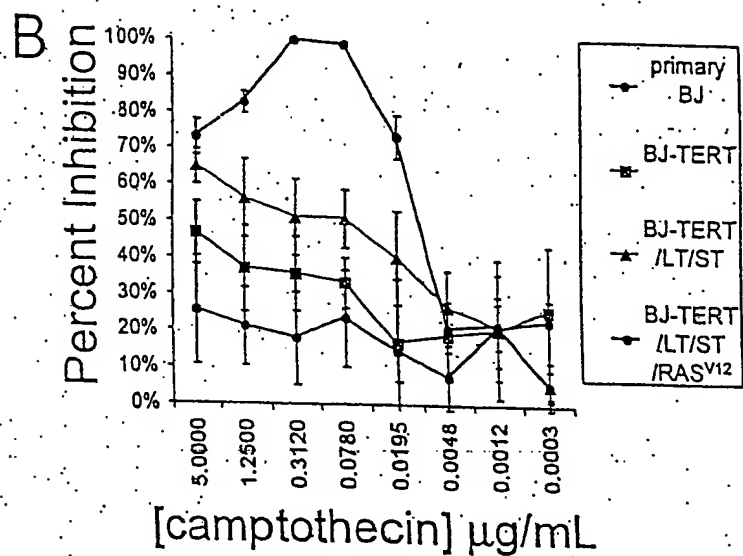
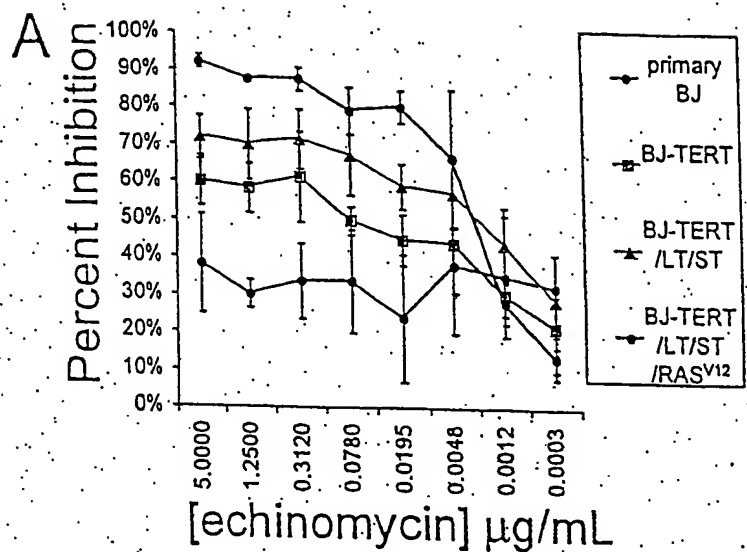


Figure 3

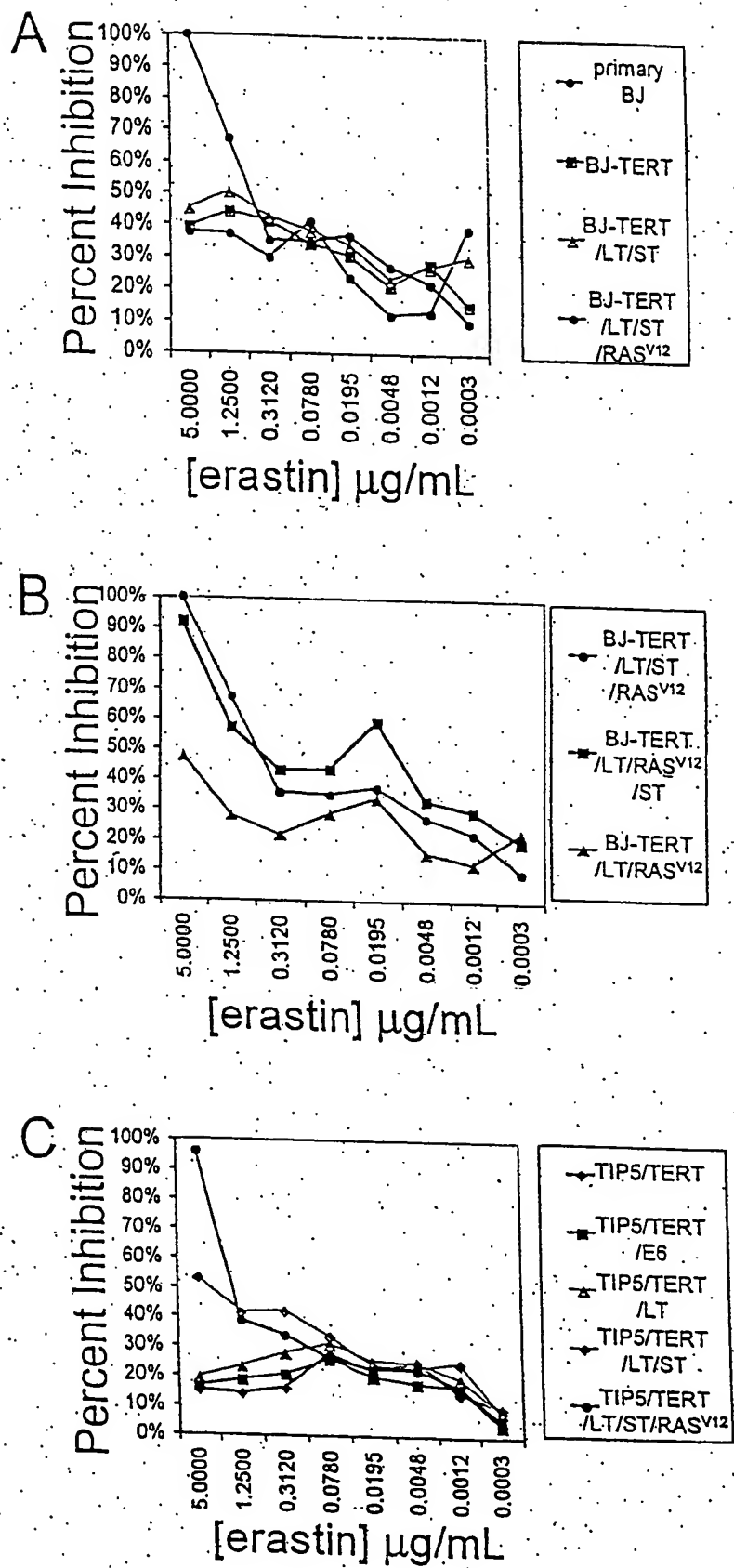


Figure 4

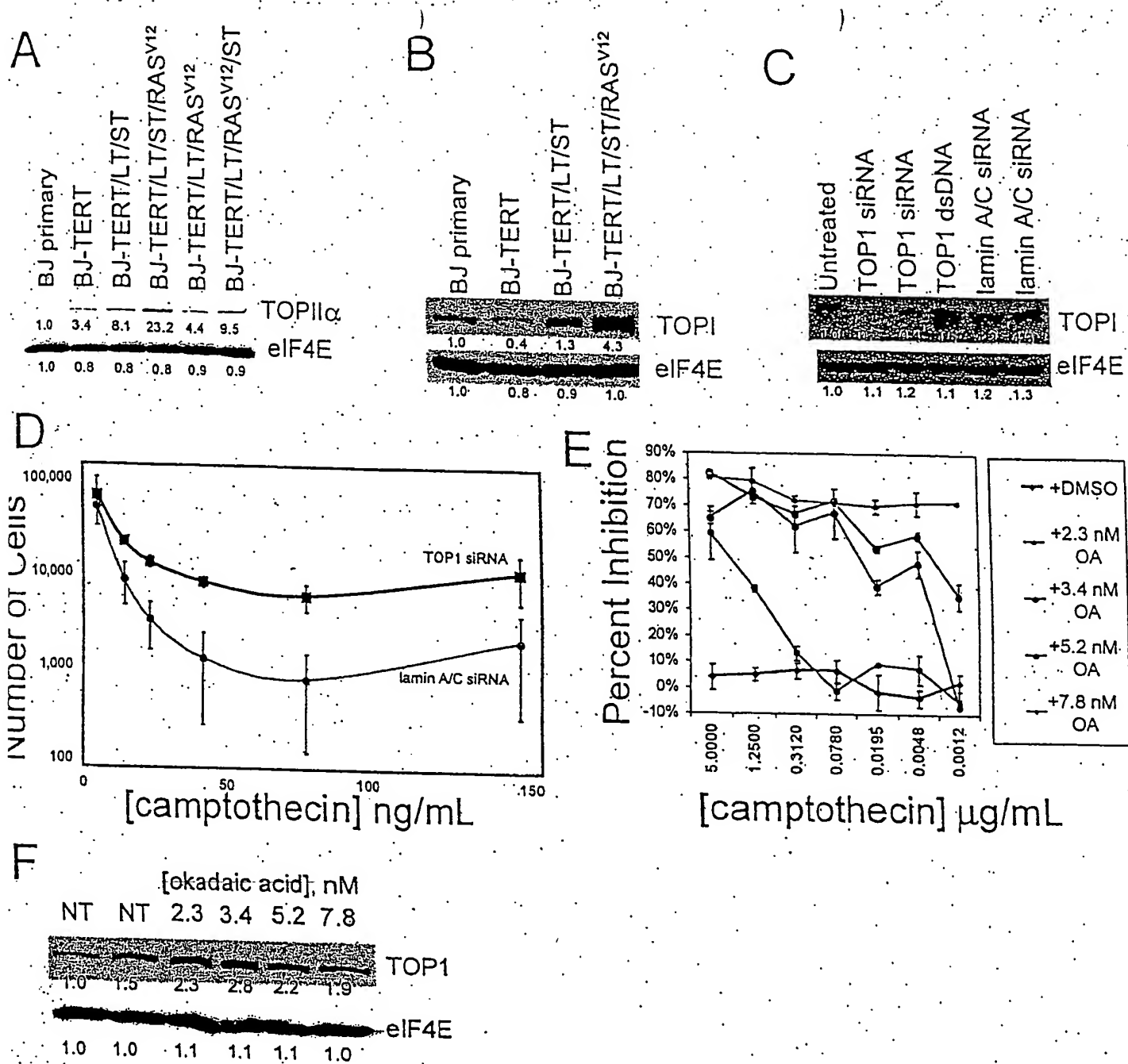


Figure 5

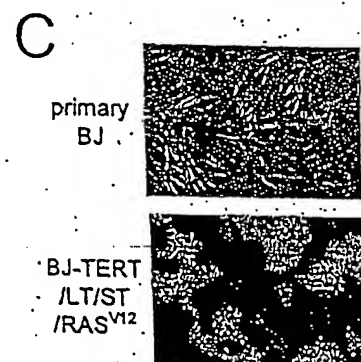
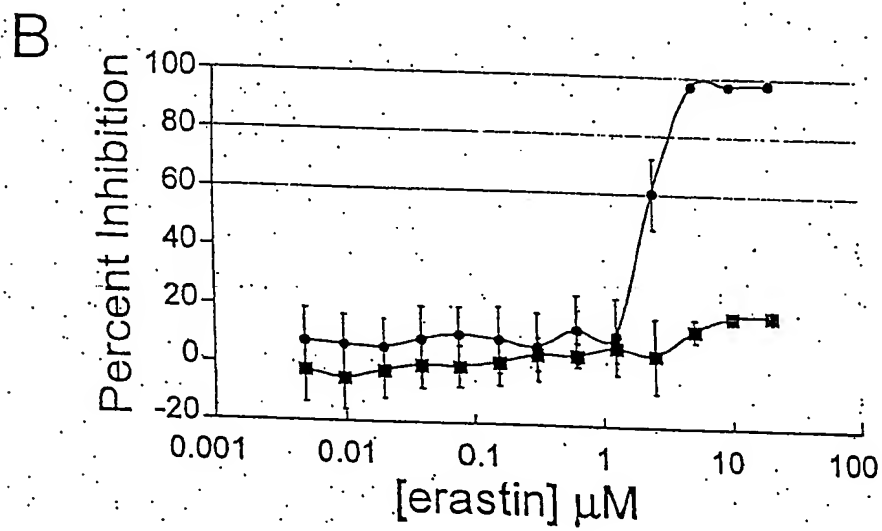
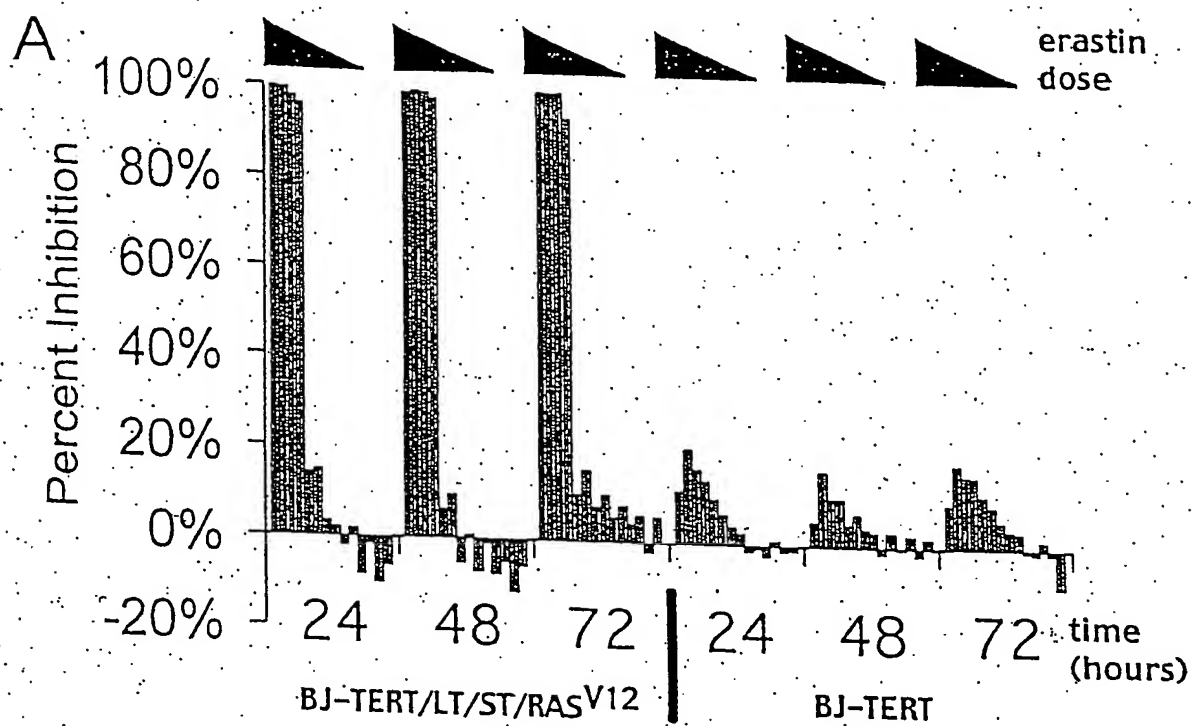


Figure 6

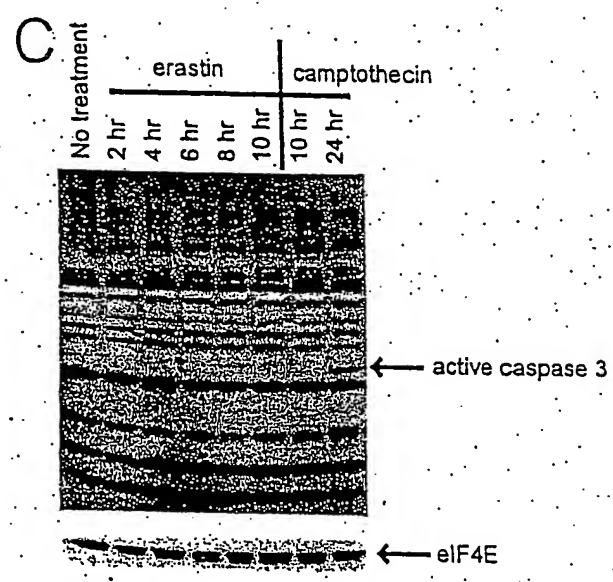
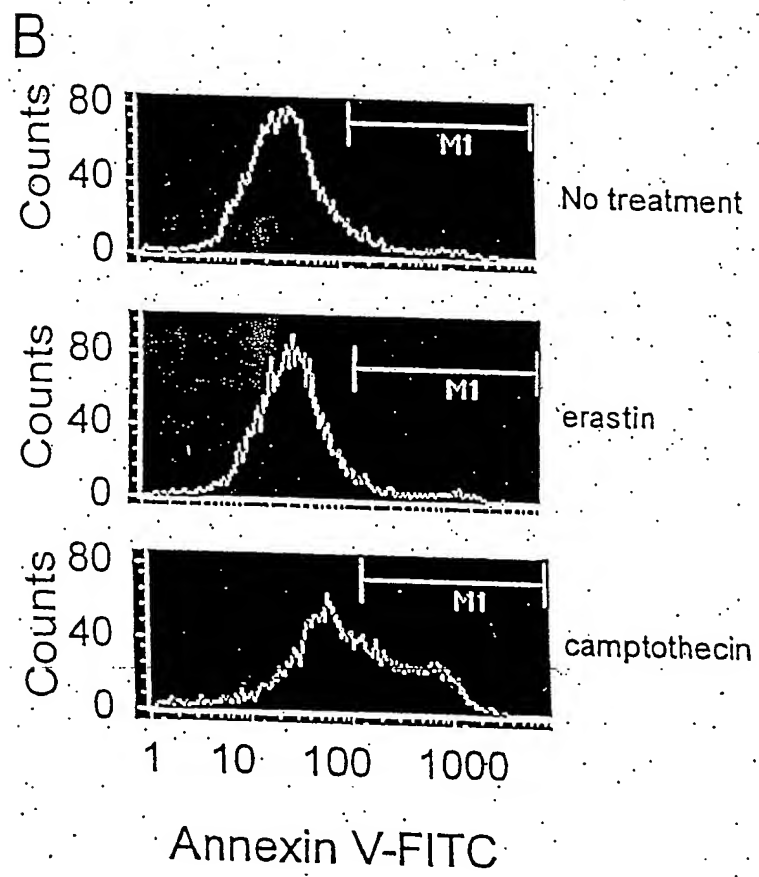
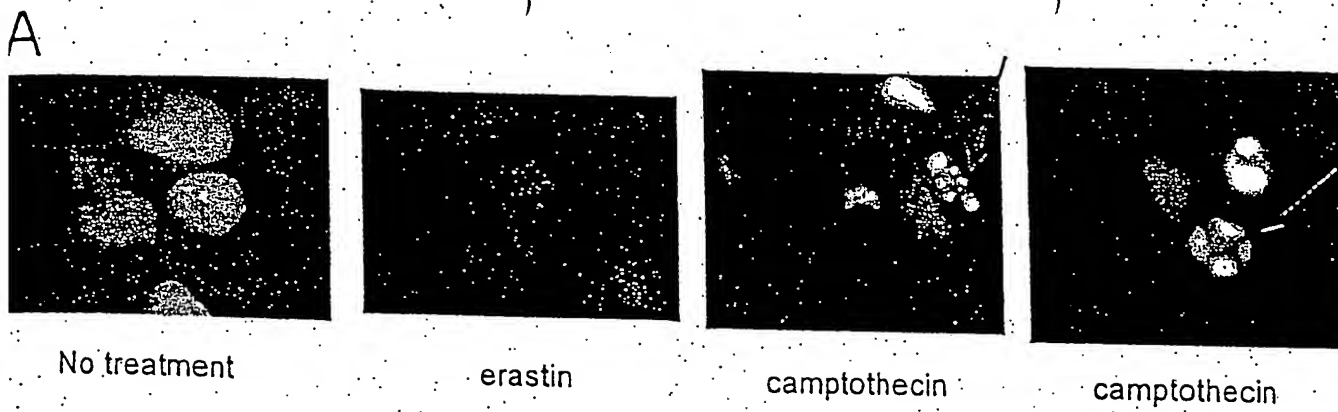


Figure 7

Structure Activity Relationship for Erastin

- Tested 135 analogs of erastin for activity and selectivity (tumor vs. normal)
- 134 were inactive
- 1 was active and selective, but less potent than erastin

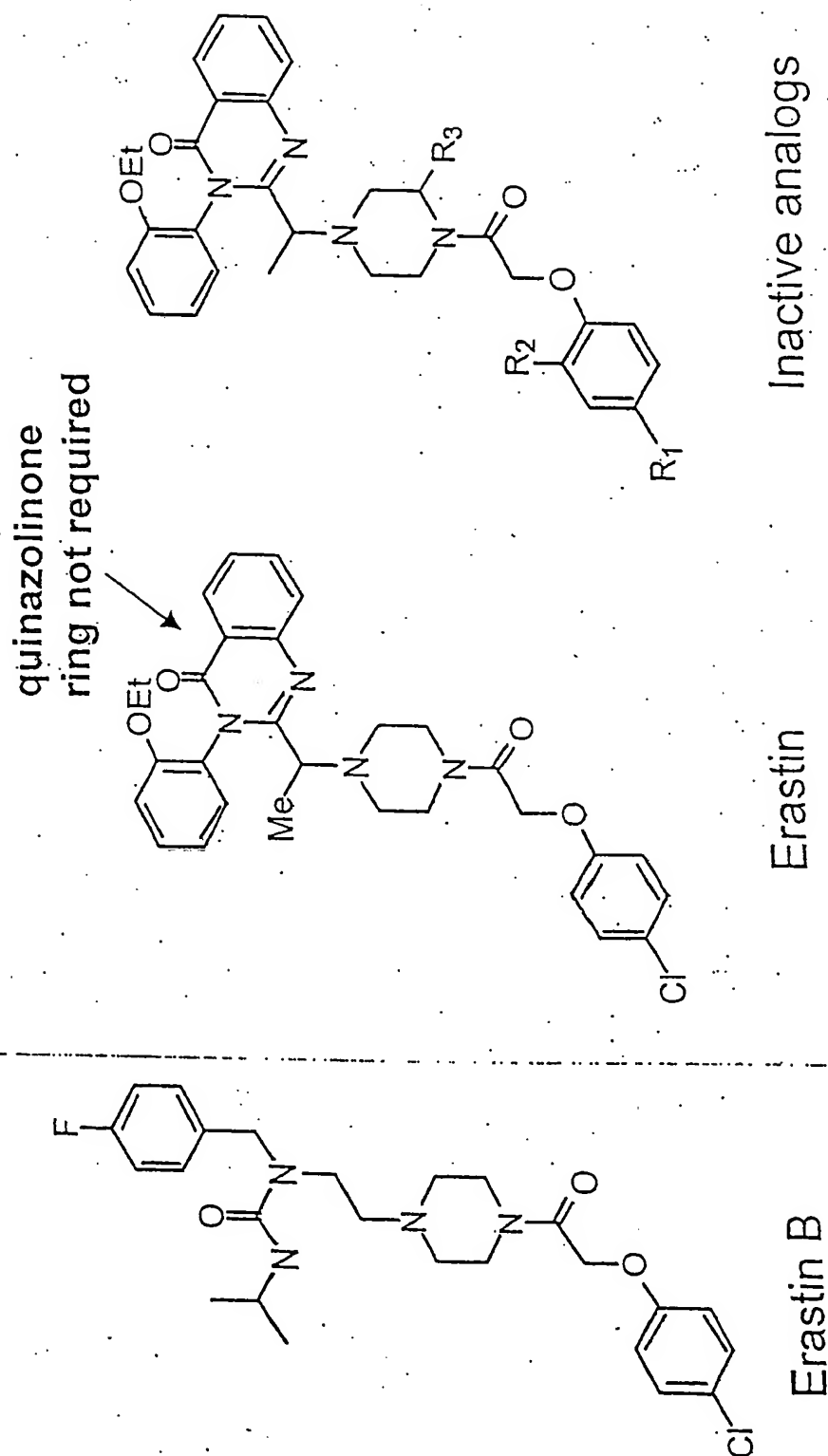


Figure 8

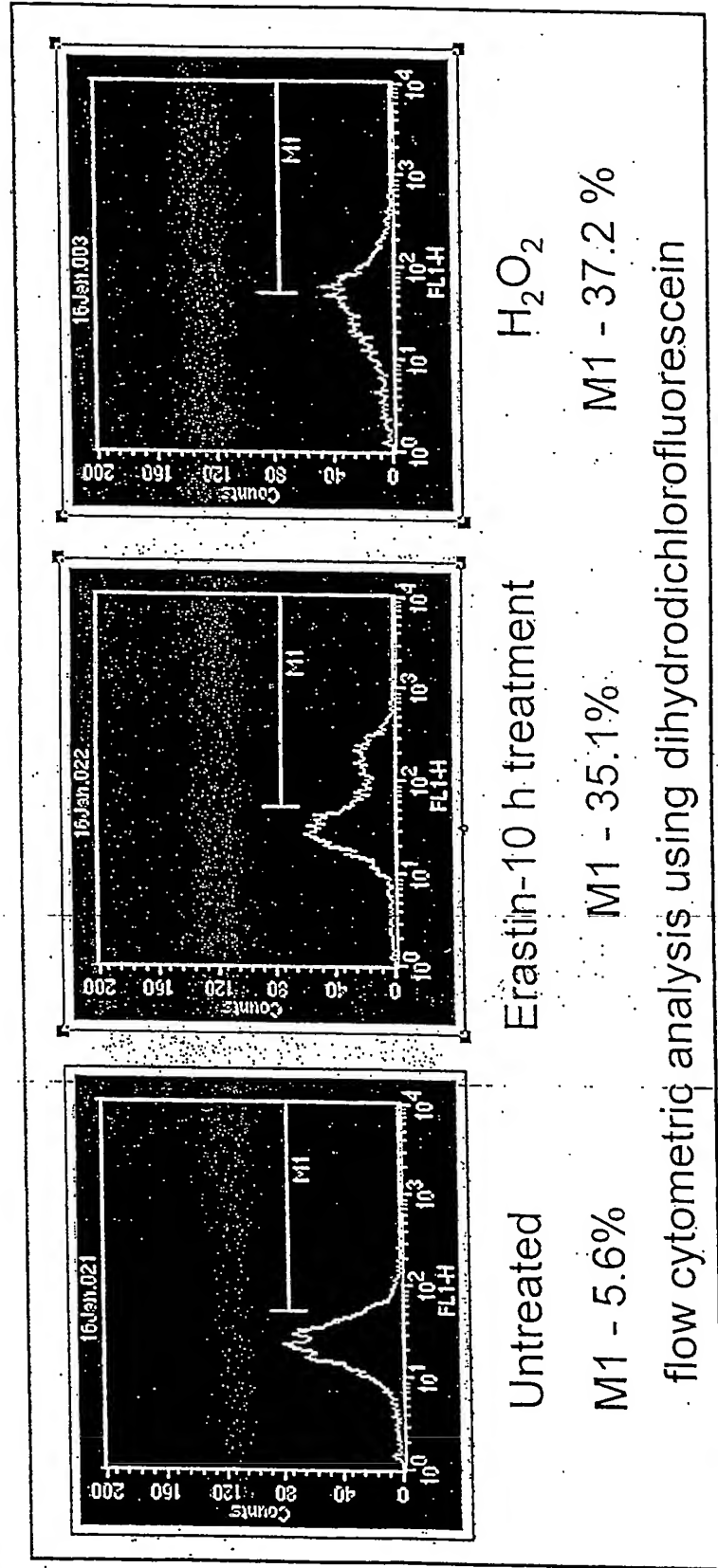
*Nuclei Are Intact in Erastin-treated
Tumor Cells*

9 μ M erastin 18 hrs



Figure 9

Erastin Induces the Formation of Reactive Oxygen Species



flow cytometric analysis using dihydrodichlorofluorescein

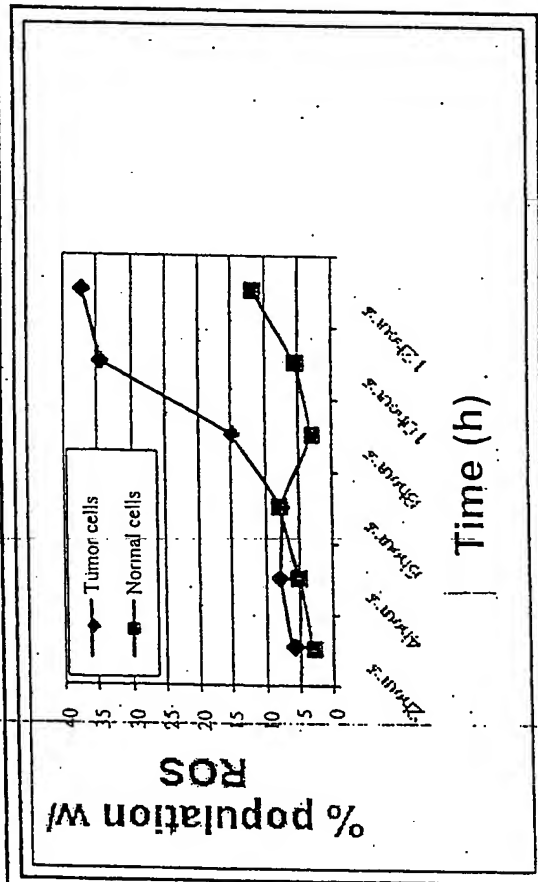
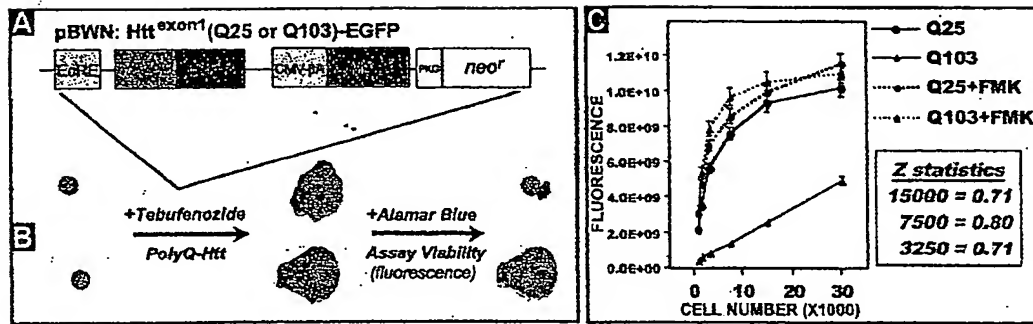
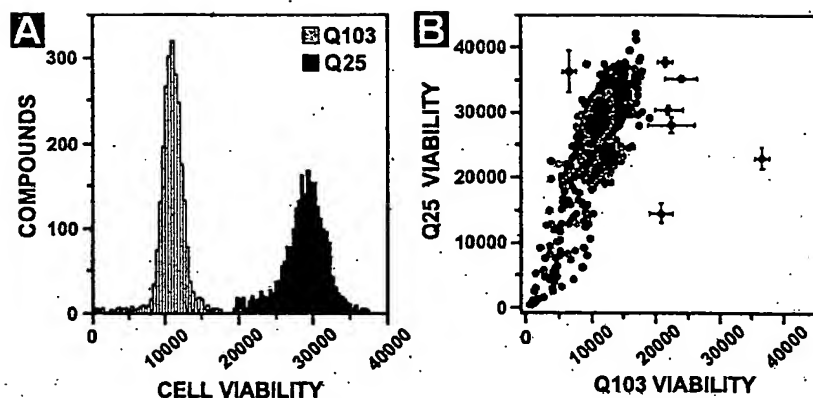


Figure 10



Modeling Htt-polyQ neurotoxicity in PC12 cells. (A) Inducible construct for production of Htt-EGFP fusion proteins. Rat neuronal PC12 cells are transfected with Htt-exon-1 constructs containing either 25 (Q25) or 103 (Q103) polyglutamine repeats (mixed CAG/CAA). (B) Cartoon of Htt-exon-1 expression in PC12 cells and screening assay for cell viability using Alamar Blue. Induction of Htt-Q103 expression leads to the formation of perinuclear cytoplasmic inclusions (or aggresomes) of the fusion protein followed by cytotoxicity after 48 hours. Expression of Htt-Q25 remains diffuse throughout the cytoplasm and is not cytotoxic. (C) Quantification of Htt-Q25 and Htt-Q103 cell viability as a measure of Alamar Blue fluorescence. Note addition of the general caspase inhibitor (BOC-D-FMK, 50 μ M) rescues Htt-Q103 toxicity after a 72 hour induction with tebufenozide (Z-statistics calculated for 15000, 75000, and 3250 cells, yellow box).

Figure 11



Primary screening results of the ACL library (2036 compounds) using the Q25-Htt-exon-1 and Q103-Htt-exon-1 PC12 cell lines. The plots at left show two representations of this data set. **(A)** Histogram plot showing cell viability of Q25-Htt and Q103-Htt expressing PC12 cells after 72 hours in culture with compounds (binning interval is 400 fluorescence units). Cell viability, represented along the horizontal axis, was quantified by Alamar Blue fluorescence. **(B)** Scatter plot showing cell viability (Q25 versus Q103) following the 72 hour incubation in the presence of each compound (4 µg/ml).

Color legend as follows: **Black**=2029 compounds having either no effect, cytotoxic effect, or slight rescue of cell viability; **Red**= top 6 compounds that rescued Q103-induced cell death; **Blue**= one compound that specifically enhanced Q103-mediated cytotoxicity; **Green**= overlay scatter of 400 control wells without compound (average standard deviation of control wells: Q25=3845, Q103=517). Each data point in plot (B) was calculated from an average of three replicates. The standard deviations (error bars) are shown for the 7 highlighted compounds.

Figure 12

Figure 13. Effect of cell density on coefficient of variation (CV)

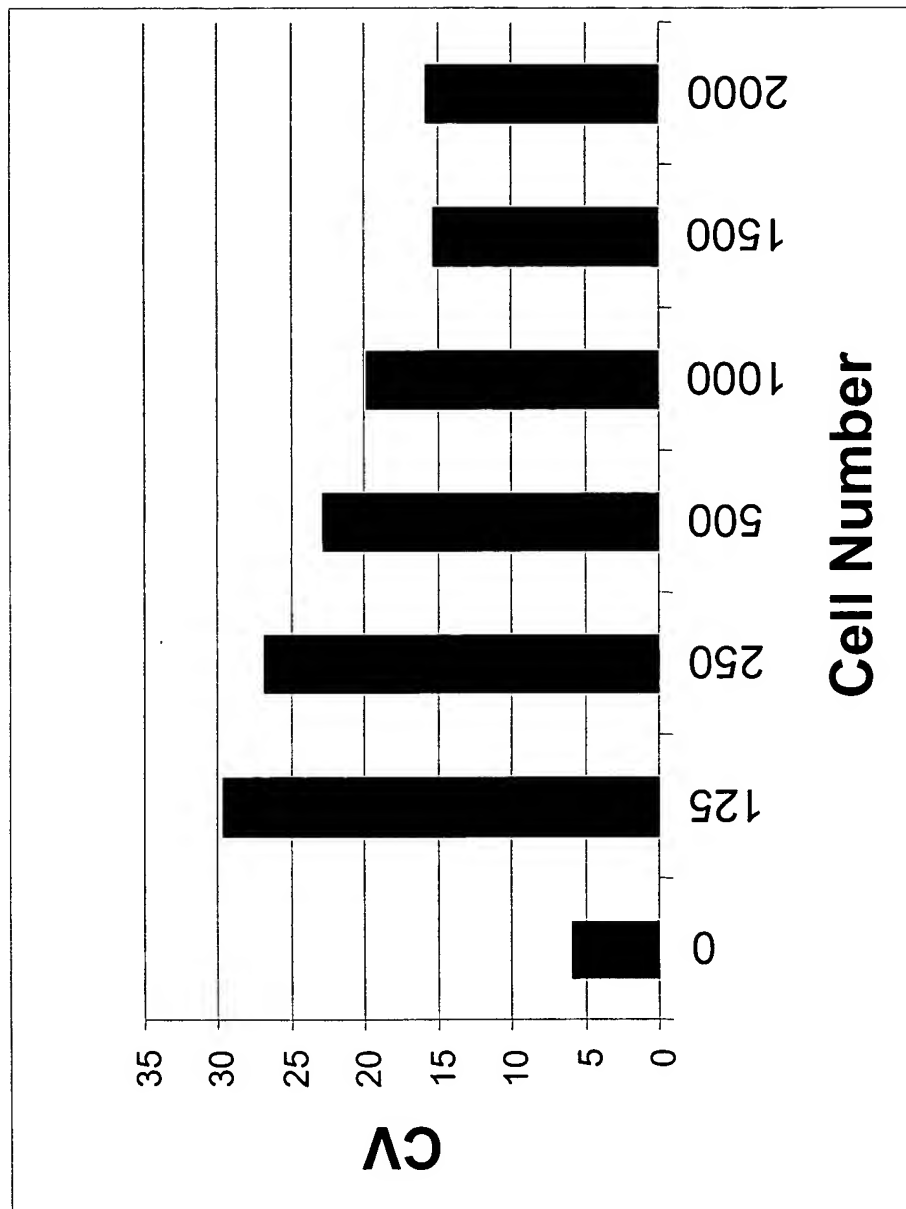


Figure 13

**Figure 14. Dose-Response for Suppressor of Mutant
Huntingtin-Induced Toxicity**

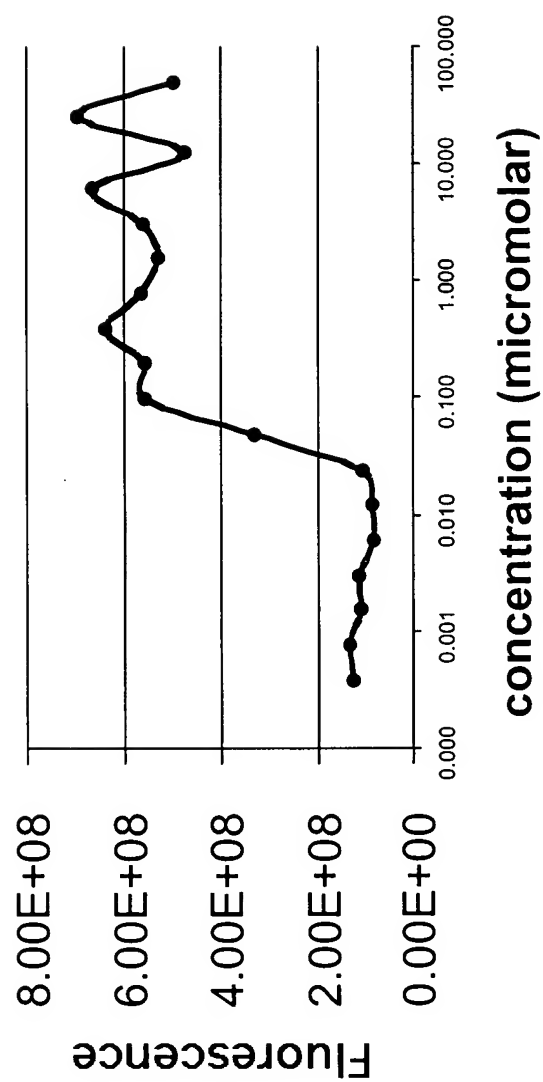
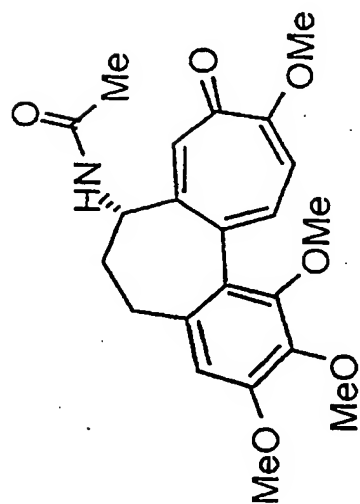
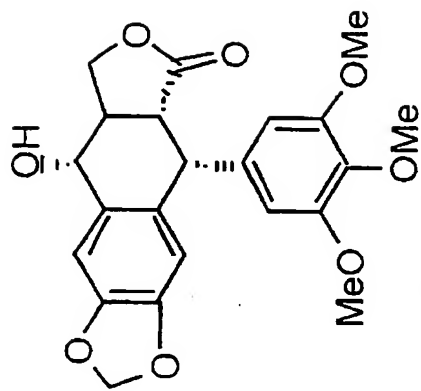


Figure 14

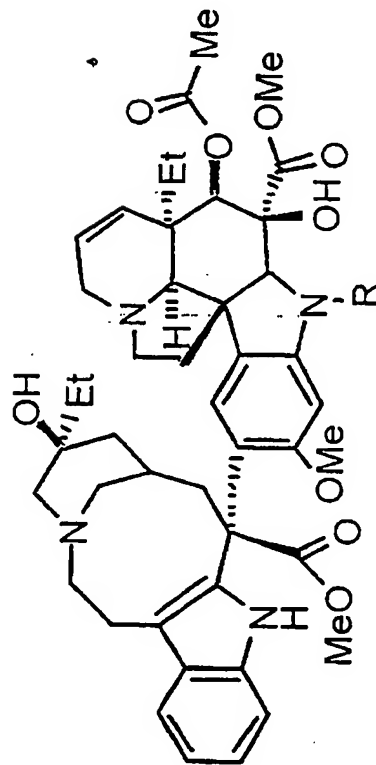
Tubulin Inhibitors Suppress Mutant Huntingtin-Induced Cell Death



colchicine



podophyllotoxin



vincristine, vinblastine

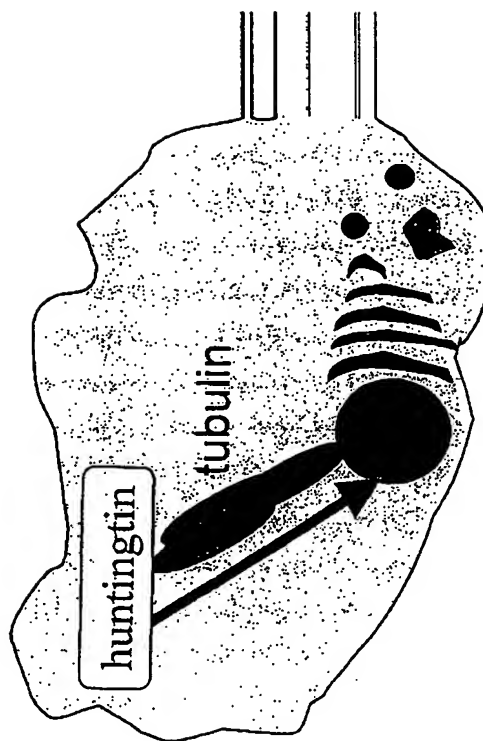


Figure 15